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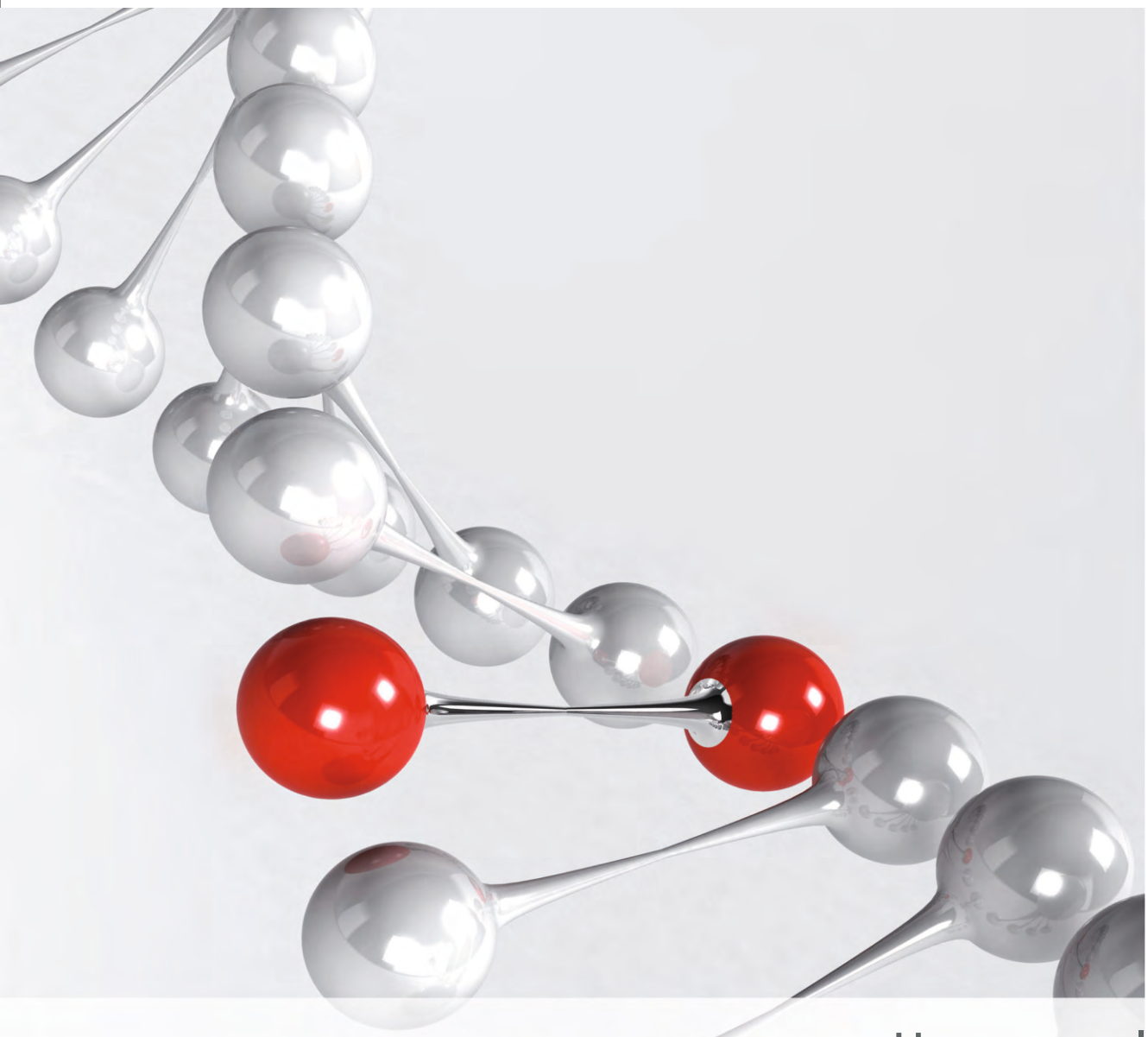

User manual

PSP® Spin Stool DNA Kit/ PSP® Spin Stool DNA Plus Kit

for (collection, storage, stabilization) and purification of total DNA from fresh or frozen stool samples (including Stool Collection Tubes with Stool DNA Stabilizer)

CE
IVD

REF 1038100x00
1038110x00

STRATEC Molecular GmbH, D-13125 Berlin

Instruction for the PSP® Spin Stool DNA Kit

The **PSP® Spin Stool DNA Kit** provides fast and easy purification of total DNA from max. 200 mg of fresh or frozen stool samples using the Invisorb® technology. The purified DNA is of high quality and well suited for use in *in-vitro* diagnostic analysis.

Instruction for PSP® Spin Stool DNA *Plus* Kit

The **PSP® Spin Stool DNA *Plus* Kit** is an integrated system for collection, transportation and storage of stool samples and subsequent DNA purification. The kit has been designed for isolation of DNA from pathogenic microorganisms, as well as for isolation of DNA from the host organism. Furthermore, it is possible to extract nucleic acids from food and feed residues of plant or animal origin from the stool sample. The purified DNA is ideal for reliable use in PCR and other downstream enzymatic reactions.

The kits are neither validated for the isolation of genomic DNA from cultured or isolated cells, from tissues, swabs, dried blood stains, or cell free body fluids, like synovial fluid and urine or the purification of RNA.

The application of the kits for isolation and purification of viral DNA has not been evaluated.

CE **IVD** Compliance with EU Directive 98/79/EC on *in vitro* medical devices.

Not for in-vitro diagnostic use in countries where the EU Directive 98/79/EC on in vitro medical devices is not recognized.

Trademarks: Invisorb®, PSP®, InviMag®, Eppendorf®. Registered marks, trademarks, etc. used in this document, even when not specifically marked as such, are not to be considered unprotected by law.

The Invisorb® technology is covered by patents and patent applications: US 6,110363, US 6,043,354, US 6,037,465, EP 0880535, WO 9728171, WO 9534569, EP 0765335, DE 19506887, DE 10041825.2, WO 0034463.

Invisorb®, PSP® and InviMag® are registered trademarks of STRATEC Biomedical AG.

The PCR process is covered by US Patents 4,683,195, and 4,683,202 and foreign equivalents owned by Hoffmann-La Roche AG.

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Kit components of PSP® Spin Stool DNA Kit

Store all kit components at room temperature (RT).
Store diluted Proteinase K at –20°C, but repeated freezing and thawing will reduced the activity dramatically. Divide the Proteinase K into aliquots and store at –20°C.

	5 extractions	**50 extractions**	**250 extractions**
Catalog No	1038100100	1038100200	1038100300
Lysis Buffer P	3 x 2 ml	120 ml	2 x 160
Zirconia Beads II	1 vial	2 vial	8 vials
InviAdsorb	5	50	5 x 50
Proteinase K	for 250 µl working solution	for 1.5 ml working solution	for 5 x 1.5 ml working solution
Binding Buffer A	2 x 1 ml (ready to use)	4 ml (final volume 15 ml)	2 x 9 ml (final volume 2 x 30 ml)
Wash Buffer I	15 ml (ready to use)	30 ml (final volume 60 ml)	80 ml (final volume 160 ml)
Wash Buffer II	15 ml (ready to use)	18 ml (final volume 60 ml)	2 x 45 ml (final volume 2 x 150 ml)
Elution Buffer	2 ml	15 ml	60 ml
2.0 ml Safe-Lock-Tubes	10	2 x 50	10 x 50
RTA Spin Filter Set	5	50	5 x 50
RTA Receiver Tubes	2 x 5	2 x 50	10 x 50
1.5 ml Receiver Tubes	2 x 5	2 x 50	10 x 50
Manual	1	1	1
Initial steps	Add 250 µl ddH_2O to **Proteinase K,** mix thoroughly until completely dissolving and store the tube at -20°C! Incubate the needed amount of **Elution Buffer** at 70°C in a **t**hermomixer	Add 11 ml 99.7% Isopropanol to the **Binding Buffer A.** Mix by intensive shaking by inverting for 1 min. Shortly before use mix by inverting several times. Add 1.5 ml ddH_2O to **Proteinase K,** mix thoroughly until completely dissolving and store the tube at -20°C! Add 30 ml of 96 - 100% ethanol to the bottle **Wash Buffer I** mix thoroughly and always keep the bottle firmly closed! Add 42 ml of 96 - 100% ethanol to the bottle **Wash Buffer II**, mix thoroughly and always keep the bottle firmly closed! Incubate the needed amount of **Elution Buffer** at 70°C in a thermomixer	Add 21 ml 99.7% Isopropanol to each **Binding Buffer A.** Mix by intensive shaking by inverting for 1 min. Shortly before use mix by inverting several times. Add 1.5 ml ddH_2O to each tube **Proteinase K**, mix thoroughly until completely dissolving and store the tube at -20°C! Add 80 ml of 96 - 100% ethanol to the bottle **Wash Buffer I** and always keep the bottle firmly closed! Add 105 ml of 96 - 100% ethanol to each bottle **Wash Buffer II**, mix thoroughly and always keep the bottle firmly closed! Incubate the needed amount of **Elution Buffer** at 70°C in a thermomixer

Kit components of PSP® Spin Stool DNA *Plus* Kit

Store all kit components at room temperature (RT).
Store diluted Proteinase K at –20°C, but repeated freezing and thawing will reduced the activity dramatically. Divide the Proteinase K into aliquots and store at –20°C.

	5 extractions	50 extractions	250 extractions
Catalog No	1038110100	1038110200	1038110300
Stool Collection Tubes with Stool DNA Stabilizer	5	2 x 25	10 x 25
InviAdsorb	5	50	5 x 50
Zirconia Beads II	1 vial	2 vial	8 vials
Proteinase K	for 250 µl working solution	for 1.5 ml working solution	for 5 x 1.5 ml working solution
Binding Buffer A	3 x 1 ml (ready to use)	9 ml (final volume 30 ml)	36 ml (final volume 120 ml)
Wash Buffer I	15 ml (ready to use)	30 ml (final volume 60 ml)	80 ml (final volume 160 ml)
Wash Buffer II	15 ml (ready to use)	18 ml (final volume 60 ml)	2 x 45 ml (final volume 2 x 150 ml)
Elution Buffer	2 ml	15 ml	60 ml
2.0 ml Safe-Lock-Tubes	10	2 x 50	10 x 50
RTA Spin Filter Set	5	50	5 x 50
RTA Receiver Tubes	2 x 5	2 x 50	10 x 50
1.5 ml Receiver Tubes	2 x 5	2 x 50	10 x 50
Manual	1	1	1
Initial steps	Add 250 µl ddH_2O to **Proteinase K,** mix thoroughly until completely dissolving and store the tube at -20°C! Incubate the needed amount of **Elution Buffer** at 70°C in a thermomixer	Add 21 ml 99.7% Isopropanol to the **Binding Buffer A.** Mix by intensive shaking by inverting for 1 min. Shortly before use mix by inverting several times. Add 1.5 ml ddH_2O to **Proteinase K,** mix thoroughly until completely dissolving and store the tube at -20°C! Add 30 ml of 96 - 100% ethanol to the bottle **Wash Buffer I** and always keep the bottle firmly closed! Add 42 ml of 96 – 100 % ethanol to the bottle **Wash Buffer II**, mix thoroughly and always keep the bottle firmly closed! Preheat needed amount of **Elution Buffer** at 70°C in a thermomixer	Add 84 ml 99.7% Isopropanol to the **Binding Buffer A.** Mix by intensive shaking by inverting for 1 min. Shortly before use mix by inverting several times. Add 1.5 ml ddH_2O to each tube **Proteinase K**, mix thoroughly until completely dissolving and store the tube at -20°C! Add 80 ml of 96 - 100% ethanol to the bottle **Wash Buffer I** and always keep the bottle firmly closed! Add 105 ml of 96 - 100% ethanol to each bottle **Wash Buffer II**, mix thoroughly and always keep the bottle firmly closed! Preheat needed amount of **Elution Buffer** at 70°C in a thermomixer

Symbols

Manufacturer

Lot number

Catalogue number

Expiry date

Consult operating instructions

Temperature limitation

Do not reuse

Storage

All buffers and kit contents of the **PSP® Spin Stool DNA Kit** and the **PSP® Spin Stool DNA *Plus* Kit** except dissolved **Proteinase K** should be stored at room temperature (RT) and are stable for at least 12 months under these conditions.

Room temperature (RT) is defined as range from 15 - 30°C.

Proteinase K: Dissolved **Proteinase K** must be stored at -20°C. Repeated freezing and thawing will reduce the activity of **Proteinase K** dramatically. Dividing Proteinase K into aliquots and store at -20°C.

Wash Buffer I and **II: Wash Buffer** charged with ethanol should be stored at room temperature and should be sealed appropriately. If there are any precipitates within the provided solutions redissolve these precipitates by careful warming up to room temperature (up to 30°C).

Quality control and product warranty

STRATEC Molecular warrants the correct function of the **PSP® Spin Stool DNA Kit** and the **PSP® Spin Stool DNA *Plus* Kit** for applications as described in this manual. Purchaser must determine the suitability of the Product for its particular use. Should any Product fail to perform the applications as described in the manual, STRATEC Molecular will check the lot and if STRATEC Molecular investigates a problem in the lot, STRATEC Molecular will replace the Product free of charge.

STRATEC Molecular reserves the right to change, alter, or modify any Product to enhance its performance and design at any time.

In accordance with STRATEC Molecular's ISO 9001-2000 and ISO EN 13485 certified Quality Management System the performance of all components of the **PSP® Spin Stool DNA Kit** and the **PSP® Spin Stool DNA *Plus* Kit** have been tested separately against predetermined specifications routinely on lot-to-lot to ensure consistent product quality.

If you have any questions or problems regarding any aspects of **PSP® Spin Stool DNA Kit** and the **PSP® Spin Stool DNA *Plus* Kit** or other STRATEC Molecular products, please do not hesitate to contact us. A copy of STRATEC Molecular's terms and conditions can be obtained upon request or are presented at the STRATEC Molecular webpage.

For technical support or further information please contact:

from Germany +49-(0)30-9489-2901/ 2910
from abroad +49-(0)30-9489-2907
or contact your local distributor.

Intended use

The **PSP® Spin Stool DNA Kit** has been designed for fast and efficient purification of genomic and microbial DNA from up to 400 mg fresh and frozen human or animal stool samples or from other sample types with high concentrations of PCR inhibiting components.

The purified DNA can be used for in-vitro diagnostic analysis.

The **PSP® Spin Stool DNA *Plus* Kit** is an integrated system for collection, transportation and storage of fecal samples and subsequent DNA purification. The kit has been designed for isolation of DNA from pathogenic microorganisms, as well as for isolation of DNA from the host organism. Furthermore, it is possible to extract nucleic acids from food and feed residues of plant or animal origin from the stool sample.

The protocols for the isolation and all buffers are optimized for a high yield as well as a high purity. All hands on steps are reduced to a minimum.

Any diagnostic results generated using the sample preparation procedure in conjunction with any downstream diagnostic assays should be interpreted with regard to other clinical or laboratory finding.

THE PRODUCT IS INTENDED FOR USE BY PROFESSIONALS ONLY, SUCH AS TECHNICIANS, PHYSICIANS AND BIOLOGISTS TRAINED IN MOLECULAR BIOLOGICAL TECHNIQUES. It is designed to be used with any downstream application employing enzymatic amplification or other enzymatic modifications of DNA followed by signal detection or amplification. Any diagnostic results generated by using the sample preparation procedure in conjunction with any downstream diagnostic assay should be interpreted with regard to other clinical or laboratory findings.

To minimize irregularities in diagnostic results, adequate controls for downstream applications should be used.

The kit is in compliance with EU Directive 98/79/EC on in vitro medical devices. But it is not for in-vitro diagnostic use in countries where the EU Directive 98/79/EC on in vitro medical devices is not recognized.

Product use limitation

The kit is not validated for the isolation of genomic DNA from cultured or isolated cells, from tissue, swabs, dried blood stains, or cell free body fluid, like synovial fluid and urine, or the purification of RNA. The application of the kit for isolation and purification of viral DNA has not been evaluated.

The included chemicals are only useable once.

Differing of starting material or flow trace may lead to inoperability; therefore neither a warranty nor guarantee in this case will be given, neither implied nor express.

The user is responsible to validate the performance of the STRATEC Molecular Product for any particular use. STRATEC Molecular does not provide for validation of performance characteristics of the Product with respect to specific applications. STRATEC Molecular Products may be used e.g.in clinical diagnostic laboratory systems conditioned upon the complete diagnostic system of the laboratory the laboratory has been validated pursuant to CLIA' 88 regulations in the U.S. or equivalents in other countries.

All Products sold by STRATEC Molecular are subject to extensive quality control procedures (according to ISO 9001-2000 and ISO EN 13485) and are warranted to perform as described herein. Any problems, incidents or defects shall be reported to STRATEC Molecular immediately upon detection thereof.

The chemicals and the plastic parts are for laboratory use only; they must be stored in the laboratory and must not be used for purposes other than intended.

The Product with its contents is unfit for consumption.

Safety information

When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles and avoid skin contact. Heed the legal requirements for working with biological material! For more information, please consult the appropriate material safety data sheets (MSDS). These are available online in convenient and compact PDF format at **www.stratec.com** for each STRATEC Molecular kit and whose kit component.

If the buffer bottles are damaged or leaking, wear gloves and protective goggles when discarding the bottles in order to avoid any injuries.

STRATEC Molecular has not tested the liquid waste generated by the **PSP® Spin Stool DNA Kit** and the **PSP® Spin Stool DNA *Plus* Kit** procedures for residual infectious materials. Contamination of the liquid waste with residual infectious materials is highly unlikely, but cannot be excluded completely. Therefore, liquid waste must be considered infectious and must be handled and discarded according to local safety regulation.

Below is listed European Community risk and safety phrases for the components of the **PSP® Spin Stool DNA Kit** and of the **PSP® Spin Stool DNA *Plus* Kit** to which they apply

Lysis Buffer P

danger

H319 P305-351-338

Wash Buffer I

warning

H302-312-332-412 EUH032 P273

Proteinase K

danger

H315-319-334-335 P280-305-351-338-310-405

H319:	Causes serious eye irritation.
H315:	Causes skin irritation.
H334:	May cause allergy or asthma symptoms or breathing difficulties if inhaled.
H335:	May cause respiratory irritation.
H302:	Harmful if swallowed.
H312:	Harmful in contact with skin.
H332:	Harmful if inhaled.
H412:	Harmful to aquatic life with long lasting effects.
EUH032:	Contact with acids liberates very toxic gas.
P233:	Keep container tightly closed.
P305+P351+P338:	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P280:	Wear protective gloves/protective clothing/eye protection/face protection.
P310:	Immediately call a POISON CENTER or doctor/physician.
P405:	Store locked up.
P273:	Avoid release to the environment.

Emergency medical information can be obtained 24 hours a day from infotrac:

outside of USA: **1 – 352 – 323 – 3500**
in USA : **1 – 800 – 535 – 5053**

Product characteristic of the PSP® Spin Stool DNA Kit

Starting material	Yield	Time	Ratio
max. 200 mg fecal sample	up to 50 µg (depends on starting material)	about 45 min (incl. lysis time)	A_{260} : A_{280} 1,4 – 1,8

The **PSP® Spin Stool DNA Kit** allows rapid and efficient isolation of high quality DNA from up to 200 mg of fresh or frozen stool sample. Stool samples typically contain many compounds that can degrade DNA and inhibit downstream enzymatic reactions. To ensure the removal of these contaminants the **PSP® Spin Stool DNA Kit** contains tubes with **InviAdsorb** and optimized essential washing conditions to remove all potent inhibitors very efficiently. So the simple PSP® Spin procedure yields pure DNA ready to use in less than 1h.

A rigorous prelysis step using **Zirconia Beads II** with optimized prelysis buffer under high temperatures is followed by a preincubation of the sample with **InviAdsorb** to remove PCR inhibitors. Undissolved particles and PCR inhibitors bound to **InviAdsorb** are removed by a centrifugation step. The following **Proteinase K** digestion ensures high yields also from gram positive bacteria. Stool contains a range of DNA e.g. host DNA from colon epithelial cells, parasite DNA, bacterial DNA, DNA from food or DNA from gastrointestinal pathogens. The choise of different lysis conditions allows the enrichment or a reduction of the content of bacterial DNA in the total DNA. All impurities are removed very efficiently during washing steps and the purified DNA is eluted directly in a low-salt buffer.

No phenol/chloroform extraction or ethanol precipitation is necessary. The kit provides reproducible recovery rates of highly purified DNA, ready to use in any downstream application. The isolated DNA can be stored at -20°C for later use.

Due to the high purity, the isolated total DNA is suitable for a broad panel of downstream applications (see below) or can be stored at -80°C for subsequent use.

- PCR* applications
- RFLP-analysis
- Hybridization
- Genetic typing
- Pathogen typing
- Mutation analysis

No toxic or hazardous chemicals like phenol/chloroform or ß-Mercaptoethanol are used.
Traditional time-killing procedures can be replaced using the **PSP® Spin Stool DNA Kit.**

To increase robustness of PCR assays using DNA isolated from stool samples, the addition of BSA to a final concentration of 0.1 µg/ µl to the PCR mixtures is recommended. In DNA eluates from feces, the ratio of target DNA to background DNA is often very low.

*) The PCR method is covered by U.S. Patents 4,683,195 and 4,683,202 owned by Hoffmann-LaRoche Inc. The purchase of the **PSP® Spin Stool DNA Kit** cannot be construed as an authorization or implicit licence to practice PCR under any patents held by Hoffmann-LaRoche Inc.

Product characteristic of the PSP® Spin Stool DNA *Plus* Kit

Starting material	Yield	Time	Ratio
1.4 ml Stool DNA Stabilizer with stool homogenate	up to 50 µg (depends on starting material)	about 45 min (incl. lysis time)	A_{260} : A_{280} 1,4 – 1,8

The **PSP® Spin Stool DNA *Plus* Kit** combines the collection of stool samples, the storage and stabilization of the stool specimen without any degradation of the DNA with a very efficient and fast isolation of high quality total DNA. The **PSP® Spin Stool DNA *Plus* Kit** can be used for isolation of DNA from pathogenic microorganism, as well as for isolation of DNA from host organism.

The **PSP® Spin Stool DNA *Plus* Kit** uses a stabilizing reagent, the **Stool DNA Stabilizer,** which enables the storage of the stool samples after collection without cooling under ambient temperature for at least 3 days.
The system combines the use of the Stool Collection Tubes prefilled with the **Stool DNA Stabilizer** for collection, storage and stabilization of the stool specimen without any degradation of the DNA during transportation and the prelysis of bacteria with a very efficient and fast isolation of high quality DNA from stool sample. In addition to the inactivation of DNases, the **Stool DNA Stabilizer** preserves the microorganism titer. Furthermore, the **Stool DNA Stabilizer** enables prelysis of gram positive or negative bacteria. For the DNA extraction process only a small amount of the total volume will be used, the residual sample can be used for further extractions or a long term storage at -20°C.

Stool samples typically contain many components that can degrade DNA and inhibit down stream enzymatic reactions. Therefore the **PSP® Spin Stool DNA *Plus* Kit** contains **InviAdsorb**, a reagent that efficiently adsorbs these components at the beginning of the purification process and additionally optimized essential washing conditions for the final removal of the last traces of all potent inhibitors. (See more to the procedure at page 11 and19.)

Due to the high purity, the isolated DNA is suitable for a broad panel of downstream applications (see below) or can be stored at –20°C for later use.

- PCR applications
- RFLP analysis
- Hybridization
- Genetic typing
- Pathogen typing
- Mutation analysis
- Paternity testing

To purify high molecular weight total DNA using magnetic beads, STRATEC Molecular offers different kits in single sample format as well as 15 well or in 96 format, the **InviMag® Stool DNA Mini Kit** for use on the **InviMag Rack,** the **InviMag® Stool DNA Mini Kit KFmL** and **KF96** for use on the KingFisher workstations.

*) The PCR method is covered by U.S. Patents 4,683,195 and 4,683,202 owned by Hoffmann-LaRoche Inc. The purchase of the **PSP® Spin Stool DNA *Plus* Kit** cannot be construed as an authorization or implicit licence to practice PCR under any patents held by Hoffmann-LaRoche Inc.

Principle and procedure

The **PSP® Spin Stool DNA Kit** procedure comprises following steps:

- lysis of sample
- removal of PCR inhibitors
- protein digestion
- binding the nucleic acids to the membrane of a spin column
- washing of the spin column and hereby elimination of contaminants and ethanol
- elution of the nucleic acids

After homogenization of the sample in the **Lysis Buffer P** which inactivates DNases, the human cells and the bacterial cell wall will be lysed differently (depending on the temperature profile). The lysate will be mixed with **InviAdsorb** and most of the PCR inhibiting components will be removed followed by a protein digestion. After lysis the DNA binds to the membrane, contaminations and enzyme inhibitors are efficiently removed during the following three washing steps and highly purified DNA is eluted in **Elution Buffer** or water.

The **PSP® Spin Stool DNA *Plus* Kit** procedure comprises some additional steps likes:

- sample collection
- DNA stabilization in the sample
- followed by all steps from the **PSP® Spin Stool DNA Kit** procedure

After sample collection and homogenization of the sample in the Stool Collection Tube containing the DNA stabilizing **Lysis Buffer** which inactivates DNases, the human and bacterial cells will be lysed differently (depending from the temperature profile). The DNA is stable for more than 3 days and can be transported to the lab without degradation, prelysing bacteria and stopping further bacterial grow. The lysate will be mixed later with **InviAdsorb** and the most PCR inhibiting components will be removed, followed by a protein digestion. After lysis the DNA binds to the membrane, contaminations and enzyme inhibitors are efficiently removed during the following three wash steps and highly purified DNA is eluted in **Elution Buffer** or water.

This manual contains 4 protocols.

Sampling and storage of starting material

PSP® Spin Stool DNA Kit
The collected fresh stool sample can be stored at ambient temperature for at least 1-2 hours at RT, but the high content of DNases realize quickly a DNA digestion and degradation. The sample should be quickly added to the **Lysis Buffer P** or can be stored frozen at – 20°C for weeks.

PSP® Spin Stool DNA *Plus* Kit
The storage of fresh samples in **Stool DNA Stabilizer** provided in the Stool Collection Tubes, allow storage at RT for about 3 days. The storage of fresh samples in **Stool DNA Stabilizer** will lead to less degraded DNA and a better yield of bacterial pathogens with difficult to lyse cell walls. Storage time below 3 days has no influence on the quality or the amount of host cell DNA. The collected sample in Stool DNA Stabilizer can also be used immediately for the isolation of DNA after collection.
The collected sample can be refrigerated at –20°C immediately after collection or after storage at ambient temperature for a later use (for example for a second DNA isolation).

STRATEC Molecular will be released of its responsibilities if other sample materials than described in the Intended Use are processed or if the sample preparation protocols are changed or modified.

Procedure

Lysis
Stool samples are lysed in **Lysis Buffer P** under denaturing conditions at high temperatures. Human cells lyse efficiently at RT, bacterial cells and those of other pathogens in the stool sample are efficiently lysed by incubation at 95°C. This is recommended for detection of cells that are difficult to lyse (e.g. gram positive bacteria).

Note: *The total DNA concentration in the lysate will be increased 3-5 fold by lysis at 95°C and the ratio of non human to human DNA will increase.*

Removal of PCR inhibitors
After lysis procedure DNA damaging substances and PCR inhibitors which are present in the feces are adsorbed efficiently to the **InviAdsorb** matrix. **InviAdsorb** is provided very convenient in safe lock tubes and the lysate must only be mixed with the matrix. The bound contaminations and cell debris are pelleted by centrifugation. The supernatant contains the precleaned DNA.

Protein digestion
Proteinase K is added to the supernatant to digest and degrade proteins during the incubation at 70°C.

Binding of total DNA
After adding **Binding Buffer A** to the supernatant, the mixture is transferred to the spin columns and nucleic acids are bound to the membrane of the RTA Spin Filter during a brief centrifugation step.
Optimal salt concentrations and pH conditions in the lysate ensure that remains of digested proteins and other contaminations, which can inhibit downstream enzymatic reactions, are not retained on the Invisorb membrane.

Removing residual contaminants
DNA bound to the Invisorb membrane is washed in three centrifugation steps. Contaminants are efficiently and completely removed using **Wash Buffer I** and **II**, while the nucleic acids remain bound to the membrane.

Elution
The nucleic acids are eluted in low salt buffer from the membrane using 100 - 200 µl **Elution Buffer**. The eluted nucleic acids are ready to use in different subsequent tests.

Yield and quality of total DNA

The amount of purified DNA in the **PSP® Spin Stool DNA Kit** procedure from feces depends on the health status of the donor, bacteria content, sample source, transport, storage, and age. A typical yield is 10 – 80 µg, a typical DNA concentration is 50 – 300 ng/ µl. Yield and quality of isolated genomic DNA is suitable for any molecular-diagnostic detection system. The diagnostic tests should be performed according to manufacturers' specifications.

Important notes

Important points before starting a protocol

Immediately upon receipt of the Product, inspect the Product and its components as well as the package for any apparent damages, correct quantities and quality. If there are any unconformities you have to notify STRATEC Molecular in writing with immediate effect upon inspection thereof. If buffer bottles are damaged, contact the STRATEC Molecular Technical Services or your local distributor. In case of liquid spillage, refer to “Safety Information” (see page 7). Do not use damaged kit components, since their use may lead to poor kit performance.

- Always change pipet tips between liquid transfers. To avoid cross-contamination, we recommend the use of aerosol-barrier pipet tips.
- All centrifugation steps are carried out at room temperature.
- When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles.
- Discard contaminated gloves.
- Do not combine components of different kits unless the lot numbers are identical.
- Avoid microbial contamination of the kit reagents.
- To minimize the risk of infections from potentially infectious material, we recommend working under laminar air-flow until the samples are lysed.
- This kit should only be used by trained personnel.

Preparing reagents and buffers for the PSP® Spin Stool DNA Kit and PSP® Spin Stool DNA *Plus* Kit

1. Adjust the thermomixer to 70°C.
2. Dissolve Proteinase K in ddH_2O.
3. Warm up the needed amount of **Elution Buffer** to 70°C, (100 - 200 µl **Elution Buffer** are needed per sample).
4. Heat heating blocks (e.g. thermomixer) to 70°C and 95 °C.
5. Label the needed amount of 2.0 ml RTA Spin Filter Sets.
6. Label the needed amount of 1.5 ml Receiver Tubes (per sample: 1 Receiver Tube), add the needed amount of ethanol to the **Wash Buffer I** and **II** (see Kit contents, page 3 and 4).

5 total DNA extractions:
add 250 µl ddH_2O to Proteinase K, mix thoroughly until completely dissolving and store the tube at -20°C **Binding Buffer A, Wash Buffer I** and **II** are ready to use
50 total DNA extractions:
PSP® Spin Stool DNA Kit: add 11 ml 99.7% Isopropanol to the **Binding Buffer A.** Mix by intensive shaking by inverting for 1 min. **PSP® Spin Stool DNA *Plus* Kit:** add 21 ml 99.7% Isopropanol to the **Binding Buffer A.** Mix by intensive shaking by inverting for 1 min. Shortly before use mix by inverting several times. add 1.5 ml ddH_2O to **Proteinase K**, mix thoroughly until completely dissolving and store the tube at -20°C add 30 ml 96-100% ethanol to the bottle **Wash Buffer I** add 42 ml 96-100% ethanol to each bottle **Wash Buffer II** mix thoroughly and always keep the bottle firmly closed
250 total DNA-extractions:
PSP® Spin Stool DNA Kit: add 21 ml 99.7% Isopropanol to each **Binding Buffer A.** Mix by intensive shaking by inverting for 1 min. **PSP® Spin Stool DNA *Plus* Kit:** add 84 ml 99.7% Isopropanol to the **Binding Buffer A.** Mix by intensive shaking by inverting for 1 min. Shortly before use mix by inverting several times. add 1.5 ml ddH_2O to **Proteinase K**, mix thoroughly until completely dissolving and store the tube at -20°C add 80 ml 96-100% ethanol to each bottle **Wash Buffer I** add 105 ml 96-100% ethanol to each bottle **Wash Buffer II** mix thoroughly and always keep the bottle firmly closed

Equipment and reagents to be supplied by user

When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDS). (See our webpage: **www.stratec.com**)

- Microcentrifuge
- Thermomixer (for 95°C)
- Measuring cylinder (250 ml)
- Disposable gloves
- Pipet with tips
- Reagents reservoirs for multichannel pipets
- 96 - 100% ethanol
- ddH_2O
- Vortexer or other homogenizer
- Isopropanol

*The **PSP® Spin Stool DNA Kit** and the **PSP® Spin Stool DNA *Plus* Kit** are validated with 2-Propanol; Rotipuran >99.7%, p.a., ACS, ISO (Order no. 6752) from **Carl Roth**

*** Possible suppliers for Isopropanol:**

Carl Roth
2-Propanol
Rotipuran >99.7%, p.a., ACS, ISO
Order no. 6752

Applichem
2-Propanol für die Molekularbiologie
Order no. A3928

Sigma
2-Propanol
Order no. 59304-1L-F

Important indications

1. The kit procedure is also suitable for purifying DNA from very small amounts of starting material. If the sample has less than 5 ng DNA (>1.000 copies), 3-5 µg Carrier (a homopolymer such as poly–dA, poly-dT or genomic DNA) should be added to the starting material. Ensure that the Carrier DNA does not interfere with downstream application. In order to prevent any interference of the carrier with the downstream application, a RNA carrier can be used. This can be removed by RNase digestion. The carrier should be added to the lysis buffer before preparation or to the stabilization buffer, never add to the stool directly.

2. Invisorb® RTA Spin filter can also purify low amounts of RNA besides DNA. For the elimination of RNA (if necessary) add 20 µl RNase A (10 mg/ml) before adding the **Binding Buffer A**. Vortex briefly and incubate the sample at room temperature for 5 minutes. Then go on as described in the protocol.

Elution of DNA

- For downstream applications, that require small starting volumes, a more concentrated eluate may increase assay sensitivity. The elution can be done by using a lower volume of **Elution Buffer** (down to 60 µl). This may result in a higher concentration of DNA. But lower volumes of **Elution Buffer** will decrease the yield of DNA.
- The final volume of eluate recovered may be up to 5 µl less than the volume of elution buffer applied to the spin filter.
- If low concentrated TRIS-buffer affects sensitive downstream applications, use distilled sterile water for elution. However, ensure that the pH of the water is at least 7,0 (deionized water from certain sources can be acidic). DNA stored in water is subjected to degradation by acid hydrolysis.
- Eluting twice with each 100 µl **Elution Buffer** is also possible and gives slightly higher yield of DNA.

Handling of the RTA Spin Filter Set

Due to the sensitivity of DNA amplification technologies, the following precautions are necessary:

- to avoid cross-contamination between sample preparation when handling RTA Spin Filter Set carefully apply the sample or solution to the RTA Spin Filter Set, pipet the sample into the filter without wetting the rim of the column
- always change pipet tips between liquid transfers, we recommend the use of aerosol-barrier pipet tips
- avoid touching the RTA Spin Filter membrane with the pipet tip

Scheme of the PSP® Spin Stool DNA Kit

Please read protocols prior the start of the preparation carefully

transfer 200 mg of the stool sample into a 2 ml Safe-Lock-Tube,
add 1.2 ml **Lysis Buffer P**, vortex for 1 min.

for enrichment of host DNA:
incubate 10 min at RT under shaking

for enrichment of bacterial DNA:
incubate 10 min at 95°C on a thermomixer under shaking,
add 5 **Zirconia Beads II** to the homogenate and vortex for 2 min

spin down at 11.100 x g (11.000 rpm) for 1 min

transfer the supernatant to the InviAdsorb-Tube
mix it by vortexing for 15 sec.
incubate 1 min at RT
spin down for 3 min at full speed

transfer the supernatant in a new 1.5 ml Receiver Tube
centrifuge the sample again at full speed for 3 min.
transfer 400 µl of the sample supernatant in a new 1.5 ml Receiver Tube

add 25 µl **Proteinase K** to the same 2.0 ml Safe-Lock-Tube
mix shortly by vortexing
incubate for 10 min at 70 °C while continuously shaking on a thermomixer at 900 rpm

add 200 µl **Binding Buffer A** *(follow preparing instructions)* to the lysate
mix shortly by vortexing or pipetting up and down

transfer the whole mixture to the RTA Spin Filter
incubate for 1 min at RT
centrifuge at 11.000 x g (11.000 rpm) for 2 min
discard the filtrate and the RTA Receiver Tube

transfer the RTA Spin Filter in a new RTA Receiver Tube
pipet 500 µl **Wash Buffer I** onto the RTA-Spin Filter
centrifuge at 11.000 x g (11.000 rpm) for 1 min
discard the flow-through and the RTA Receiver Tube

put the RTA Spin Filter in a new RTA Receiver Tube
pipet 700 µl **Wash Buffer II** onto the RTA Spin Filter
centrifuge at 11.000 x g (11.000 rpm) for 1 min.
discard the flow-through and reuse the RTA Receiver Tube

to eliminate any traces of ethanol, centrifuge again for 4 min at maximum speed, discard the RTA Receiver Tube

transfer the RTA Spin Filter into a new 1.5 ml Receiver Tube
pipet 100 - 200 µl of **Elution Buffer** (preheated to 70°C) directly onto the center of the membrane of the RTA Spin Filter
incubate for 1 min at RT
centrifuge at 11.000 x g (11.000 rpm) for 1 min

discard the RTA-Spin Filter
place the eluted total DNA immediately on ice and store at –20°C

Instructions

The following notes are valid for all protocols:

Note: *The centrifugation steps were made with the **Centrifuge 5415 D from Eppendorf.** The indicated refers to this centrifuge.*

Protocol 1: Isolation of total DNA from up to 200 mg stool samples with and without enrichment of bacterial DNA

Please read protocols prior the start of the preparation and complete preparing steps!!

Attention: *Please be aware, that you have to prepare the **Binding Buffer A** – see instruction page: 13*

***Important Note*:** *Please note, that the majority of extracted DNA from stool samples is of bacterial origin !*

Heat heating blocks (e.g. thermomixer) to 70°C and 95 °C

*Preheat the **Elution Buffer** to 70°C (e.g. transfer the needed volume into a tube and place it at the appropriate temperature into a thermomixer)*

1. Sample homogenization and prelysis

Weigh 200 mg of stool sample (fresh or frozen) into a 2.0 ml Safe-Lock-Tube and add 1.2 ml **Lysis Buffer P** to each stool sample .Vortex vigorously for 1 min. Even if you use less starting material, perform the protocol like described**.**

Important: *If the sample is liquid, pipet 200 µl into the 2.0 ml Safe-Lock-Tube. Cut-off the end of the pipet tip to make pipetting easier.*
If the sample is frozen, use a scalpel or spatula to scrape bits of stool into the provided 2.0 ml Safe-Lock-Tube on ice. Take care, that this samples do not thaw until Lysis Buffer P is added, otherwise the DNA in the sample may degrade. After addition of the buffer, the following steps can be performed at RT or like recommended.

Incubate the sample for 10 min at RT under continuous shaking at 900 rpm.
Centrifuge the sample at 11.000 x g (11.000 rpm) for 1 min to pellet solid stool particles.

For an enrichment of bacterial DNA:

Incubate the sample for 10 min at 95°C in a thermomixer under continuously shaking at 900 rpm.
Add 5 **Zirconia Beads II** to the homogenate and vortex for 2 min at RT.
Centrifuge the sample at 11.000 x g (11.000 rpm) for 1 min to pellet solid stool particles and beads.

Important: *The incubation step at 95°C will maximize the yield of bacterial DNA, because of a very efficient disruption of the cell wall of e.g. gram positive bacteria.*

For an enrichment of host DNA, don't perform this high-temperature step

2. Removal of PCR inhibitiors

Transfer the supernatant into an **InviAdsorb-Tube** and vortex vigorously for 15 sec.
Incubate the suspension for 1 min at room temperature. Centrifuge the sample at full speed for 3 min.

3. Second sample cleanup

Transfer the supernatant completely into a new 1.5 ml Receiver Tube. Discard the pellet. Centrifuge the sample again at full speed for 3 min.

4. Proteinase K digestion

Transfer 25 µl **Proteinase K** into a new 2.0 ml Safe-Lock-Tube and pipet 400 µl of the supernatant from step 3 to the 1.5 ml Receiver Tube containing **Proteinase K**. Mix shortly by vortexing and incubate the sample for 10 min at 70°C in a thermomixer under continuous shaking at 900 rpm.

5. Binding of the DNA

Add 200 µl **Binding Buffer A** to the lysate and mix shortly by vortexing or by pipetting up and down several times.
Transfer the mixture completely onto the membrane of the RTA Spin Filter. Incubate for 1 min at room temperature and centrifuge at 11.000 x g (11.000 rpm) for 2 min. Discard the filtrate and the RTA Receiver Tube.

6. Washing steps

Put the RTA Spin Filter in a new RTA Receiver Tube. Add 500 µl **Wash Buffer I** to the membrane of the RTA Spin Filter. Close the lid and centrifuge at 11.000 x g (11.000 rpm) for 1 min. Discard the filtrate and the RTA Receiver Tube.

Put the RTA Spin Filter in a new RTA Receiver Tube. Add 700 µl **Wash Buffer II** to the membrane of the RTA Spin Filter. Close the lid and centrifuge and centrifuge at 11.000 x g (11.000 rpm) for 1 min. Discard the filtrate and put the RTA Spin Filter back to the same RTA Receiver Tube.

7. Ethanol removal

To eliminate any traces of ethanol, centrifuge again for 4 min at maximum speed, discard the RTA Receiver Tube

8. DNA Elution

Place the RTA Spin Filter into a new 1.5 ml Receiver Tube and add 100 - 200 µl preheated (70°C) **Elution Buffer** to the sample**.** Incubate for 1 min at RT. Centrifuge at 11.000 x g (11.000 rpm) for 1 min. to elute the DNA. Finally discard the RTA Spin Filter.

Note: *The DNA can also be eluted with a lower volume of Elution Buffer (depends on the expected yield of genomic DNA). But pay attention that the minimum volume for the elution is* ***50 µl and that this volume can reduce the maximum yield.*** *If a quite large amount of DNA is expected, the volume of elution can be increased.*

Note: *For long-term storage, we recommend to keep the eluted DNA at –20°C.*

Protocol 2: Isolation of total DNA from up to 200 mg stool samples from difficult to lyse bacteria

Please read protocols prior the start of the preparation and complete preparing steps!!

Attention: *Please be aware, that you have to prepare the* ***Binding Buffer A*** *– see instruction page: 13*

Important Note**:** *To lyse some special bacteria completely, (like Mycobacteria paratuberculosis or Chlamydia) a special treatment is necessary.*

Heat heating blocks (e.g. thermomixer) to 70°C and 95 °C

Prepare a container with crushed ice

Preheat the ***Elution Buffer*** *to 70°C (e.g. transfer the needed volume* into a tube and place it at the appropriate temperature into a thermomixer)

1. Sample homogenization and prelysis

Weigh 200 mg of stool sample (fresh or frozen) into a 2.0 ml Safe-Lock-Tube and add 1.2 ml **Lysis Buffer P** to each stool sample .Vortex vigorously for 1 min.

Important: *If the sample is liquid, pipet 200 µl into the 2.0 ml Safe-Lock-Tube. Cut the end of the pipet tip to make pipetting easier.*

Incubate the homogenized sample for 10 min at 95°C in a thermomixer under continuous shaking at 900 rpm.

Incubate the sample on ice for 3 minutes

Add 5 **Zirconia Beads II** to the homogenate

Put the sample back to the 95°C thermo block,

Incubate for further 3 min at 95°C.

Vortex the sample for 2 min.

Centrifuge the sample at 11.000 x g (11.000 rpm) for 1 min to pellet solid stool particles.

2. Removal of PCR Inhibitiors

Transfer the supernatant into an **InviAdsorb-Tube** and vortex vigorously for 15 sec.
Incubate the suspension for 1 min at room temperature. Centrifuge the sample at full speed for 3 min.

3. Second Sample Cleanup

Transfer the supernatant completely into a new 1.5 ml Receiver Tube. Discard the pellet.
Centrifuge the sample again at full speed for 3 min.

4. Proteinase K digestion

Transfer 25 µl **Proteinase K** into a new 2.0 ml Safe-Lock-Tube and pipet 400 µl of the supernatant from step 3 to the 1.5 ml Receiver Tube containing **Proteinase K**, mix shortly by vortexing and incubate the sample for 10 min at 70°C in a thermomixer under continuous shaking at 900 rpm.

5. Binding of the DNA

Add 200 µl of **Binding Buffer A** to the lysate and mix shortly by vortexing or by pipetting up and down several times.
Transfer the mixture completely onto the membrane of the RTA Spin Filter. Incubate for 1 min at room temperature and centrifuge at 11.000 x g (11.000 rpm) for 2 min. Discard the filtrate and the RTA Receiver Tube.

6. Washing Steps

Put the RTA Spin Filter in a new RTA Receiver Tube. Add 500 µl **Wash Buffer I** to the membrane of the RTA Spin Filter. Close the Close the lid and centrifuge at 11.000 x g (11.000 rpm) for 1 min. Discard the filtrate and the RTA Receiver Tube.

Put the RTA Spin Filter in a new RTA Receiver Tube. Add 700 µl **Wash Buffer II** to the membrane of the RTA Spin Filter. Close the Close the lid and centrifuge at 11.000 x g (11.000 rpm) for 1 min. Discard the filtrate and put the RTA Spin Filter back to the same RTA Receiver Tube.

7. Ethanol Removal

To eliminate any traces of ethanol, centrifuge again for 4 min at maximum speed, discard the RTA Receiver Tube

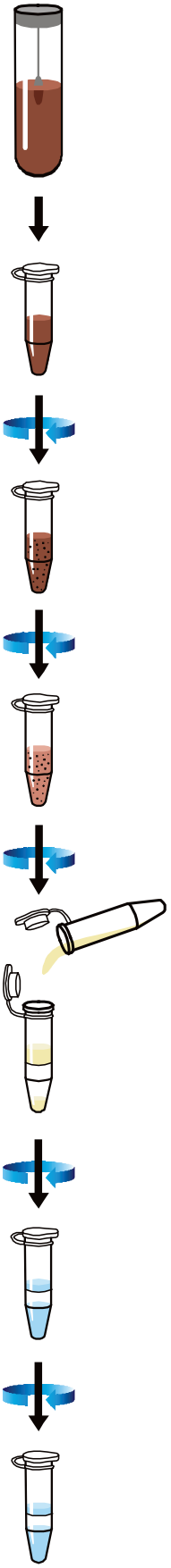
8. DNA Elution

Place the RTA Spin Filter into a new 1.5 ml Receiver Tube and add 100 - 200 µl preheated (70°C) **Elution Buffer.** Incubate for 1 min. Centrifuge at 11.000 x g (11.000 rpm) for 1 min. to elute the DNA. Finally discard the RTA Spin Filter.

Note: *The DNA can also be eluted with a lower volume of Elution Buffer (depends on the expected yield of genomic DNA). But pay attention that the minimum volume for the elution is **50 µl and that this volume can reduce the maximum yield.** If a quite large amount of DNA is expected, the volume of elution can be increased.*

Note: *For long-term storage, we recommend to keep the eluted DNA at –20°C.*

Scheme of the PSP® Spin Stool DNA *Plus* Kit



Please read protocols prior the start of the preparation carefully

collect a spoon of the stool sample
transfer stool sample into the Stool Collection Tube, close the tube
mix for a short time by shaking

transfer 1.4 ml of the stabilized stool sample (Stool DNA Stabilizer with stool specimen) into a 2.0 ml Safe-Lock Tube

for enrichment of host DNA:
incubate 10 min at RT under shaking

for enrichment of bacterial DNA:
incubate 10 min at 95°C on a thermomixer under shaking,
add 5 **Zirconia Beads II** to the homogenate and vortex for 2 min

spin down at 11.000 x g (11.000 rpm) for 1 min

transfer the supernatant to the **InviAdsorb-**Tube
mix it by vortexing for 15 sec.
incubate 1 min at RT
spin down for 3 min at full speed.

transfer the supernatant in a new 1.5 ml Receiver Tube
centrifuge the sample again at full speed for 3 min

add 25 µl **Proteinase K** in a new 2.0 ml Safe-Lock-Tube
transfer 800 µl of the sample supernatant to the same tube
mix shortly by vortexing
incubate for 10 min at 70 °C while continuously shaking on a thermomixer at 900 rpm

add 400 µl **Binding Buffer A** *(follow preparing instructions)* to the lysate
mix shortly by vortexing or pipetting up and down

transfer the whole mixture **in two steps** to the RTA Spin Filter
incubate for 1 min at RT
centrifuge at 11.000 x g (11.000 rpm) for 2 min
discard the filtrate and the RTA Receiver Tube

transfer the RTA Spin Filter in a new RTA Receiver Tube
pipet 500 µl **Wash Buffer I** onto the RTA-Spin Filter
centrifuge at 11.000 x g (11.000 rpm) for 1 min
discard the flow-through and the RTA Receiver Tube

put the RTA Spin Filter in a new RTA Receiver Tube
pipet 700 µl **Wash Buffer II** onto the RTA Spin Filter
centrifuge at 11.000 x g (11.000 rpm) for 1 min
discard the flow-through and reuse the RTA Receiver Tube

to eliminate any traces of ethanol, centrifuge again for 4 min at maximum speed, discard the RTA Receiver Tube

transfer the RTA Spin Filter into a new 1.5 ml Receiver Tube
pipet 100-200 µl of **Elution Buffer** (preheated to 70°C) directly onto the center of the membrane of the RTA Spin Filter
incubate for 1 min at RT
centrifuge at 11.000 x g (11.000 rpm) for 1 min; discard the RTA-Spin Filter;
place the eluted total DNA immediately in a refrigerator or store it at –20°C

Instructions

The following notes are valid for all protocols:

Note: *The centrifugation steps were made with the **Centrifuge 5415 D from Eppendorf.** The indicated **rpm amounts** are referring to this centrifuge.*

Protocol 1: Collection of the stool sample and stabilization

Please read protocols prior the start of the preparation and complete preparing steps!!

Note: *The **Stool Collection Tubes** contains 8 ml of **Stool DNA Stabilizer**. That is a new developed buffer formulation which enables the prelysis and stabilization of the DNA for at least 3 days at ambient temperature. The **Stool DNA Stabilizer** is very successful even if bacterial pathogens should be detected, which are difficult to lyse because of the structure of their cell walls.*

1. Open the **Stool Collection Tube** and collect a spoon of the stool sample.
2. Transfer the spoon with the stool sample back into the **Stool Collection Tube** and close the tube very tight.
3. Mix the sample for a short time by shaking. That will lead to homogenization of the stool sample.

Important Notes: *The collected sample can be stored at ambient temperature for at least 3 days. The storage under **Stool DNA Stabilizer** will lead to a better yield of bacterial pathogens with difficult to lyse cell walls. Storage time has no influence on the quality or the amount of host cell DNA.*

The collected sample can also be used immediately after collection for the isolation of DNA.

The collected sample can be frozen at –20°C immediately after collection or after storage at ambient temperature for a later use (for example for a second DNA isolation).

Protocol 2: Isolation of total DNA from 1.4 ml stabilized stool homogenate with and without enrichment of bacterial DNA

Please read protocols prior the start of the preparation and complete preparing steps!!

Attention: *Please be aware, that you have to prepare the* ***Binding Buffer A*** *– see instruction page: 13*

***Important Note*:** *Please note, that the extracted DNA from stool sample is by the majority from bacterial origin !*

Heat heating blocks (e.g. thermomixer) to 70°C and 95 °C

Preheat the ***Elution Buffer*** *to 70°C (e.g. transfer the needed volume into a tube and place it at the appropriate temperature into a thermomixer)*

1. Sample Homogenization and Prelysis

Transfer 1.4 ml of the collected and well homogenized stool sample (Stool DNA Stabilizer with stool specimen) after storage or directly after collection into the 2.0 ml Safe-Lock Tube.

Centrifuge the sample at 11.000 x g (11.000 rpm) for 1 min to pellet solid stool particles. This will lead to a reduced amount of extracted total DNA, but is not influencing the amount of human DNA

For an enrichment of bacterial DNA:

Incubate the sample for 10 min at 95°C in a thermomixer under continuously shaking at 900 rpm.
Add 5 **Zirconia Beads II** to the homogenate and vortex for 2 min at RT.
Centrifuge the sample at 11.000 x g (11.000 rpm) for 1 min to pellet solid stool particles and beads.

Important Note: *The incubation step at 95°C will lead to maximize the amount of bacterial DNA, because of a very efficient destruction of the cell wall of e.g. gram+ bacteria.*

For an enrichment of host DNA, don't perform this high-temperature step

2. Removal of PCR Inhibitiors

Transfer the supernatant into an **InviAdsorb-Tube** and vortex vigorously for 15 sec.
Incubate the suspension for 1 min at room temperature. Centrifuge the sample at full speed for 3 min.

3. Second Sample Cleanup

Transfer the supernatant completely into a new 1.5 ml Receiver Tube. Discard the pellet.
Centrifuge the sample again at full speed for 3 min.

4. Proteinase K digestion

Transfer 25 µl **Proteinase K** into a new 2.0 ml Safe-Lock-Tube and pipet 800 µl of the supernatant from step 3 to the 1.5 ml Receiver Tube containing **Proteinase K**, mix shortly by vortexing and incubate the sample for 10 min at 70°C in a thermomixer under continuous shaking at 900 rpm.

5. Binding of the DNA

Add 400 µl of **Binding Buffer A** to the lysate and mix shortly by vortexing or by pipetting up and down several times.
Transfer the mixture **in two steps** onto the membrane of the RTA Spin Filter. Incubate for 1 min at room temperature and centrifuge at 11.000 x g (11.000 rpm) for 2 min. Discard the filtrate and the RTA Receiver Tube.

6. Washing Steps

Put the RTA Spin Filter in a new RTA Receiver Tube. Add 500 µl **Wash Buffer I** to the membrane of the RTA Spin Filter. Close the lid and centrifuge at 11.000 x g (11.000 rpm) for 1 min. Discard the filtrate and the RTA Receiver Tube.

Put the RTA Spin Filter in a new RTA Receiver Tube. Add 700 µl **Wash Buffer II** to the membrane of the RTA Spin Filter. Close the lid and centrifuge at 11.000 x g (11.000 rpm) for 1 min. Discard the filtrate and put the RTA Spin Filter back to the same RTA Receiver Tube.

7. Ethanol Removal

To eliminate any traces of ethanol, centrifuge again for 4 min at maximum speed, discard the RTA Receiver Tube

8. DNA Elution

Place the RTA Spin Filter into a new 1.5 ml Receiver Tube and add 100 - 200 µl preheated (70°C) **Elution Buffer.** Incubate for 1 min. Centrifuge at 11.000 x g (11.000 rpm) for 1 min to elute the DNA. Finally discard the RTA Spin Filter.

Note: *The DNA can also be eluted with a lower volume of Elution Buffer (depends on the expected yield of genomic DNA). But pay attention that the minimum volume for the elution is **50 µl and that this volume can reduce the maximum yield.** If a quite large amount of DNA is expected, the volume of elution can be increased.*

Note: *For long-term storage, we recommend to keep the eluted DNA at –20°C.*

Protocol 3: Isolation of total DNA from up to 400 mg stool samples from difficult to lyse bacteria

Please read protocols prior the start of the preparation and complete preparing steps!!

Attention: *Please be aware, that you have to prepare the* ***Binding Buffer A*** *– see instruction page: 13*

Important Note**:** *To lyse some special bacteria completely, (like Mycobacteria paratuberculosis or Chlamydia) a special treatment is needed*

Heat heating blocks (e.g. thermomixer) to 70°C and 95 °C

Prepare a container with crashed ice

Preheat the ***Elution Buffer*** *to 70°C (e.g. transfer the needed volume into a tube and place it at the appropriate temperature into a thermomixer)*

1. Sample Homogenization and Prelysis

Transfer 1.4 ml of the collected and well homogenized stool sample (**Stool DNA Stabilizer** with stool specimen) after storage or directly after collection into the 2.0 ml Safe-Lock Tube.

Incubate the homogenized sample for 10 min at 95°C in a thermomixer under continuous shaking at 900 rpm.

Incubate the sample on ice for 3 minutes and put the sample back to the 95°C thermo block, Incubate for further 3 min at 95°C.

Add 5 **Zirconia Beads II** to the homogenate and vortex for 2 min at RT.

Centrifuge the sample at 11.000 x g (11.000 rpm) for 1 min to pellet solid stool particles.

2. Removal of PCR Inhibitiors

Transfer the supernatant into an **InviAdsorb-Tube** and vortex vigorously for 15 sec.
Incubate the suspension for 1 min at room temperature. Centrifuge the sample at full speed for 3 min.

3. Second Sample Cleanup

Transfer the supernatant completely into a new 1.5 ml Receiver Tube. Discard the pellet.
Centrifuge the sample again at full speed for 3 min.

4. Proteinase K digestion

Transfer 25 µl **Proteinase K** into a new 2.0 ml Safe-Lock-Tube and pipet 800 µl of the supernatant from step 3 to the 1.5 ml Receiver Tube containing **Proteinase K**, mix shortly by vortexing and incubate the sample for 10 min at 70°C in a thermomixer under continuous shaking at 900 rpm.

5. Binding of the DNA

Add 400 µl of **Binding Buffer A** to the lysate and mix shortly by vortexing or by pipetting up and down several times.
Transfer the mixture **in two steps** onto the membrane of the RTA Spin Filter. Incubate for 1 min at room temperature and centrifuge at 11.000 x g (11.000 rpm) for 2 min. Discard the filtrate and the RTA Receiver Tube.

6. Washing Steps

Put the RTA Spin Filter in a new RTA Receiver Tube. Add 500 µl **Wash Buffer I** to the membrane of the RTA Spin Filter. Close lid and centrifuge at 11.000 x g (11.000 rpm) for 1 min. Discard the filtrate and the RTA Receiver Tube.

Put the RTA Spin Filter in a new RTA Receiver Tube. Add 700 µl **Wash Buffer II** to the membrane of the RTA Spin Filter. Close the lid and centrifuge at 11.000 x g (11.000 rpm) for 1 min. Discard the filtrate and put the RTA Spin Filter back to the same RTA Receiver Tube.

7. Ethanol Removal

To eliminate any traces of ethanol, centrifuge again for 4 min at maximum speed, discard the RTA Receiver Tube

8. DNA Elution

Place the RTA Spin Filter into a new 1.5 ml Receiver Tube and add 100 - 200 µl preheated (70°C) **Elution Buffer.** Incubate for 1 min. Centrifuge at 11.000 x g (11.000 rpm) for 1 min to elute the DNA. Finally discard the RTA Spin Filter.

Note: *The DNA can also be eluted with a lower volume of Elution Buffer (depends on the expected yield of genomic DNA). But pay attention that the minimum volume for the elution is **50 µl and that this volume can reduce the maximum yield.** If a quite large amount of DNA is expected, the volume of elution can be increased.*

Note: *For long-term storage, we recommend to keep the eluted DNA at –20°C.*

Additional Protocol 4: Post purification of DNA containing inhibitors

Please read protocols prior the start of the preparation and complete preparing steps!!

***Important Note*:** *Stool samples are very heterologous, depending on nutritation of the producer and source of the stool. In some cases inhibitors for downstream reactions might occur in the eluted DNA. In this case the following post purifying protocol may help*

1. Eluate adjustment

Adjust your eluate to at least 100 µl, for respective dilution take water.

2. Sephadex G50 Slurry

Make slurry of Sephadex G50 by adding water to Sephadex G50 powder und soaking until the slurry is reaching its final extension. This is dependent on the amount you are producing, it shouldn't take more than 30 minutes.

3. Adsorbtion of inhibitors

Add 1/3 of your eluate volume of slurry to the eluate. Incubate for 30 minutes under continuous shaking at room temperature (RT).

4. Removal of slurry

Centrifuge the mixture at 11.000 x g (11.000 rpm) for 1 min. Take the supernatant and transfer it to a new reaction tube, it contains you purified DNA.

This purification may be repeated once, but remember that you will loose 25% of yield during every purification step.

Troubleshooting

Problem	Comments and suggestions
Clogged RTA Spin Filter	
insufficient lysis and/ or too much starting material	increase lysis time increase centrifugation speed reduce amount of starting material
Low amount or no DNA of extracted DNA	
sample stored incorrectly	sample should be stored at 4°C or – 20°C
insufficient homogenization of stool sample in **Lysis Buffer P** or in **Stool DNA Stabilizer**	repeat the DNA purification procedure with a new sample Be sure to mix the sample and **Lysis Buffer P** or in **Stool DNA Stabilizer** until the sample is thoroughly homogenized use Zirconia Beads II and vortex for homogenization
insufficient lysis	increase lysis time reduce amount of starting material overloading of Spin Filter reduces yield!
insufficient mixing of the sample with **Binding Buffer A**	mix sample sufficient by pipetting up and down with **Binding Buffer A** prior to transfer the sample onto the RTA Spin Filter membrane
no alcohol added to the **Wash Buffer I** and **II**	check that **Wash Buffer I** and **Wash Buffer II** concentrates were diluted with correct volume of 96-100% ethanol. repeat the purification procedure with a new sample
DNA not eluted efficiently	to increase elution efficiency, pipet the preheated **Elution Buffer** onto the center of the RTA Spin Filter and incubate the column for 5 minutes at room temperature before centrifugation do the elution steps twice. take higher volume of **Elution Buffer**.
A260/A280 ratio for purified nucleic acids is low	
inefficient elimination of inhibitory substances due to insufficient mixing with the **InviAdsorb** matrix	repeat the DNA purification procedure with a new sample be sure to mix the sample and **InviAdsorb** matrix until the sample is thoroughly homogenized
insufficient mixing with **Lysis Buffer P**	repeat the procedure with a new sample be sure to mix the sample and **Lysis Buffer P** immediately and thoroughly by pulse vortexing
decreased **proteinase** activity	repeat the DNA purification procedure with a new sample and with **Proteinase K** for difficult cases use double volume **Proteinase K**
no **Binding Buffer A** added to the lysate	repeat the purification procedure with a sample
Wash Buffer I and **Wash Buffer II** prepared incorrectly	check that **Wash Buffer I** and **Wash Buffer II** were diluted with 96–100% ethanol do not use denatured alcohol, which contains other substances such as methanol or methylethylketone repeat the purification procedure with a new sample

A260/A280 ratio for purified nucleic acids is low	
Wash Buffer I and **Wash Buffer II** used in the wrong order	ensure that **Wash Buffer I and Wash Buffer II** are used in the correct order in the protocol.
protein contamination	repeat washing step with Wash Buffer I in the repeated preparation
DNA does not perform well in downstream applications	
BSA not added to PCR mixture	when using eluates in PCR, for maximum PCR robustness add BSA or I-Solution to a final concentration of 0.1 µg/µl to the PCR mixture
too much DNA used in downstream reaction	the **PSP® Spin Stool DNA Kit** and **PSP® Spin Stool DNA *Plus* Kit** purifies total DNA, which could originate from many different organisms present in the original stool sample (e.g., human, animal, plant, bacterial). If the amount of total DNA is too high, PCR could be inhibited by excess total DNA. Reduce the amount of eluate used in the downstream reaction if possible
nonspecific bands in	it is likely that only a low quantity of target downstream PCR DNA is present in stool-sample eluates, together with high amounts of background DNA.
inefficient lysis of target cells	the amount of target DNA in the eluate may be low if the target cells are difficult to lyse, as is the case with some bacteria and parasites. In future preparations, prolong incubation time of the sample at 95°C and/or add zirconia beads to the stool samples lysis mixture (see **PSP® Spin Stool DNA Kit** protocol 2, page 17.)
not enough DNA in eluate	check "**Low amount or no DNA of extracted DNA**" for possible reasons.
inhibitory substances in preparation	see "A260/A280 ratio for purified nucleic acids is low" for possible reasons. Bring the eluate volume to 200 µl .add to the supernatant 400 µl **Lysis Buffer P** and mix all with 200 µl **Binding Buffer A**. Repeat the protocol 1 from step 5 of "Isolation of total DNA from up to 400 mg stool samples with and without enrichment of bacterial DNA " (page 16). **See also protocol: Post Purification, see page 25**
residual **Wash Buffer** in the eluate	ensure that the **Wash Buffer I** and **II** are used in the correct order in the protocol. add 400 µl **Lysis Buffer P** and 200 µl **Binding Buffer A** to the eluate, and continue with step 5 of "Protocol: Isolation of total DNA from up to 400 mg stool samples with and without enrichment of bacterial DNA " (page 15.
insufficient mixing with **Lysis Buffer P or Stool DNA Stabilizer**	repeat the purification with other aliquots
reduced sensitivity of amplification reaction	determine the maximum volume of eluate amplification reaction suitable for your amplification reaction. Reduce or increase the volume of eluate added to the reaction optimize your amplification system e.g. by changing template volume

Little or no supernatant visible after initial centrifugation step insufficient centrifugal force	increase the centrifugation time proportionately if your centrifuge cannot provide 13.400 x g (12.000 rpm), e.g. instead of centrifuging for 1 minutes at 13.400 x g, centrifuge for 3 minutes at 10,000 x g)
Little or no supernatant visible after centrifugation step with InviAdsorb matrix insufficient centrifugal force	with some samples, centrifugation to precipitate the **InviAsorb** matrix may result in a pellet that is not sufficiently compact in these cases, it is recommended to increase the centrifugation time for precipitation of **InviAdsorb** matrix to 6 minutes
Precipitate after addition of Binding Buffer A	in most cases, this effect comes frm big amounts of DNA in the sample. Don't remove this precipitate and follow strictly the protocol
General handling lysate not completely passed through silica membrane cross-contamination between samples	 centrifuge for 1 minute at full speed or until all the lysate has passed through the membrane to avoid cross-contamination when handling RTA Spin columns, read "Handling of RTA Spin Filter " on page 12 repeat the purification procedure with new samples

Appendix

General notes on handling DNA

Nature of DNA

The length and delicate physical nature of DNA require careful handling to avoid damage due to shearing and enzymatic degradation. Other conditions that affect the integrity and stability of DNA include acidic and alkaline environments, high temperature, and UV irradiation. Careful isolation and handling of high molecular weight DNA is necessary to ensure it will function well in various downstream applications. Damaged DNA could perform poorly in applications such as genomic Southern blotting, long-template PCR, and construction of cosmid libraries.

Handling fresh and stored material before the extraction of DNA

For the isolation of genomic DNA from cells or tissues, use either fresh samples or samples that have been quickly frozen in liquid nitrogen and stored at -70°C. This procedure minimizes degradation of crude DNA by limiting the activity of endogenous nucleases.

Storage of DNA

Store genomic DNA at +2 to +8°C. Storing genomic DNA at -15 to -25°C can cause shearing of DNA, particularly if the DNA is exposed to repeated freeze-thaw cycles. Plasmid DNA and other small circular DNAs can be stored at +2 to +8°C or at -15 to -25°C.

Drying, dissolving and pipetting DNA

Avoid overdrying genomic DNA after ethanol precipitation. It is better to let it air dry than to use a vacuum, although vacuum drying can be used with caution. Plasmid DNA and other small circular DNAs can be vacuum-dried.

To help dissolve the DNA, carefully invert the tubes several times after adding buffer and tap the tube gently on the side. Alternatively let the DNA stand in buffer overnight at +2 to +8°C. Minimize vortexing of genomic DNA since this can cause shearing.

Avoid vigorous pipetting. Pipetting genomic DNA through small tip openings causes shearing or nicking. One way to decrease shearing of genomic DNA is to use special tips that have wide openings designed for pipetting genomic DNA. Regular pipette tips pose no problem for plasmid DNA and other small fragments.

Determination of concentration, yield, and purity of DNA

Determination of concentration, yield, and purity

DNA yields are determined from the concentration of DNA in the eluate, measured by absorbance at 260 nm. Purity is determined by calculating the ratio of absorbance at 260 nm to absorbance at 280 nm. Pure DNA has an A260/A280 ratio of 1.7–2.1. Absorbance readings at 260 nm should lie between 0.1 and 1.0 to be accurate. Sample dilution should be adjusted accordingly. Use elution buffer or water (as appropriate) to dilute samples and to calibrate the spectrophotometer. Measure the absorbance at 260 and 280 nm, or scan absorbance from 220–320 nm (a scan will show if there are other factors affecting absorbance at 260 nm). Both DNA and RNA are measured with a spectrophotometer

Ordering information

Product	Package size	Catalogue No.
PSP® Spin Stool DNA Kit	5 extractions	1038100100
PSP® Spin Stool DNA Kit	50 extractions	1038100200
PSP® Spin Stool DNA Kit	250 extractions	1038100300

Single components

Lysis Buffer P	30 ml	1038101200
Binding Buffer A	9 ml	1038102800
InviAdsorb	for 10 purifications	1038107600
Wash Buffer I	30 ml	1038103300
Wash Buffer II	18 ml	1038103400
Elution Buffer	15 ml	1038104000

Product	Package size	Catalogue No.
PSP® Spin Stool DNA *Plus* Kit	5 purifications	1038110100
PSP® Spin Stool DNA *Plus* Kit	50 purifications	1038110200
PSP® Spin Stool DNA *Plus* Kit	250 purifications	1038110300

Single components

Stool DNA Stabilizer	250 ml	1038111100
Stool Collection Tubes (with Stool DNA Stabilizer)	50 tubes	1038111200
Stool Collection Tubes (with Stool DNA Stabilizer)	250 tubes	1038111300
Binding Buffer A	9 ml	1038112800
Wash Buffer I	30 ml	1038113300
Wash Buffer II	18 ml	1038113400
Elution Buffer	15 ml	1038114000

Related products

InviMag® Stool DNA Mini Kit/ KFmL	15 purifications	2438110100
InviMag® Stool DNA Mini Kit/ KFmL	75 purifications	2438110200
InviMag® Stool DNA Mini Kit/ KFmL	300 purifications	2438110400
InviMag® Stool DNA Mini Kit/ KF96	1 x 96 purifications	7438300100
InviMag® Stool DNA Mini Kit/ KF96	5 x 96 purifications	7438300200

Possible suppliers for Isopropanol:

Carl Roth
2-Propanol
Rotipuran >99.7%, p.a., ACS, ISO
Order no. 6752

Applichem
2-Propanol für die Molekularbiologie
Order no. A3928

Sigma
2-Propanol
Order no. 59304-1L-F

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